Further investigations on fatty acids determination in amniotic fluid by means of a simplified method

by

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Fatty acids determination in amniotic fluid has been shown to be a reliable method for the prenatal evaluation of fetal lung maturity ([1, 7, 10, 11, 13]). In a previous paper a simplified technique for gas chromatographic determination of fatty acids in samples of amniotic fluid has been described and a series of preliminary results has been presented ([3]).

Warren et al. (1973 and 1974) and Moore et al. (1975) suggested to use quantitative determinations of palmitic acid. More exactly, a concentration of palmitic acid of 35 μg/ml or more indicates an absent or minimal risk of respiratory distress syndrome (RDS); between 34 and 26 μg/ml there is a moderate risk, while at 25 μg/ml or below the great majority of babies will develop a severe RDS.

The french authors ([1, 7, 11]) suggested, instead, to use palmitic acid/stearic acid ratios (= P/S) because this does not require a quantitative evaluation of fatty acids, but only a semiquantitative comparison of the areas of the two peaks of palmitic and stearic acid. The critical value of P/S ratio is 5, so that in cases with values above 5 the risk of RDS is absent or minimal, while it rises sharply as the P/S ratio decreases below 5.

For reasons of simplicity and because P/S ratios are independent of possible movements of water in and out of the amniotic compartment, we ([3]) decided to use P/S ratios instead of quantitative determinations of palmitic acid; furthermore we ([6]) were able to show that myristic acid/stearic acid ratios (M/S) could occasionally supply additional informations. In the present paper we describe a further simplification of our original method and report the results on a larger series of normal and pathological cases.

MATERIAL AND METHODS

The present investigation has been performed on 54 normal and on 30 pathological cases, the details of which are reported in table 1 and table 2. Most amniotic fluid samples were obtained by means of a Drew-Smythe catheter (high puncture of the membranes) either in early spontaneous labour or before labour when induction was planned. A few samples were obtained by means of uterine puncture at caesarean section and a few were obtained by means of transabdominal amniocentesis; obviously, in both cases the membranes were still intact. Most women were in-patients of the department of obstetrics and gynaecology of the University of Genova School of Medicine (their chart number is indicated in tables 1 and 2); a small number of women were in-patients of other hospitals (they are indicated in the tables with the abbreviation « Ext »).

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Table 1. *Details of 54 normal cases.*

Case Nr.	chart number/ year	amniotic fluid obtained by	miristic acid/ stearic acid ratio	palmitic acid/ stearic acid ratio	clinical notes	newborn		
						gesta-tional age	sex	birth weight
1	995/74	a	0.52	8.88	—	40	F	2630
2	1034/74	a	1.00	20.14	—	38	M	3210
3	1053/74	a	0.62	6.62	—	40	M	3400
4	1052/74	T.C.	1.66	15.77	Breech	37	F	3400
5	1057/74	a	1.10	10.45	—	40	M	3530
6	1091/74	a	1.16	16.00	—	42	M	3870
7	1092/74	a	0.32	5.08	—	39	F	3120
8	1216/74	a	3.00	21.87	—	41	F	3130
9	1265/74	a	0.70	5.50	—	40	F	2790
10	1379/74	a	3.50	20.22	—	41	M	3100
11	1404/74	T.C.	1.00	15.00	Previous C. Section	40	M	3530
12	55/75	a	0.58	5.50	—	41	M	3270
13	69/75	a	0.82	11.17	—	40	M	2860
14	91/75	T.C.	0.50	5.50	Breech	40	F	2980
15	121/75	a	0.72	9.50	—	40	F	3200
16	499/75	a	0.93	8.60	—	40	F	3280
17	534/75	a	0.81	10.12	—	41	F	3700
18	1160/75	a	1.25	7.00	—	40	F	3000
19	Ext. 1	a	0.79	6.76	—	40	F	3440
20	Ext. 2	a	0.21	5.18	—	39	F	3250
21	Ext. 3	a	0.76	6.85	—	40	M	3310
22	Ext. 4	a	0.78	6.96	—	40	F	3650
23	Ext. 7	a	1.04	8.55	—	40	M	3500
24	Ext. 8	T.C.	0.62	5.60	Breech	38	M	2850
25	Ext. 10	a	0.77	5.36	—	40	F	3250
26	Ext. 11	a	0.70	5.87	—	39	M	3100
27	Ext. 12	a	0.95	7.96	—	41	M	3710

28	Ext. 13	a	0.80	5.01	—	40	F	3620
29	Ext. 14	a	0.91	6.31	—	41	M	3150
30	70/76	a	0.40	5.20	—	39	F	3700
31	155/76	T.C.	1.20	8.01	Breech	39	M	3630
32	242/76	T.C.	1.29	6.09	Previous C. Section	40	F	3250
33	246/76	a	0.89	7.07	-	41	M	3010
34	221/76	a	1.01	7.57	Cerclage at 9 weeks gestation	41	M	3110
35	279/76	a	1.50	13.25	—	39	F	3380
36	305/76	a	1.46	11.70	—	39	M	3680
37	361/76	a	2.20	16.70	—	40	F	3130
38	415/76	a	2.30	18.20	Varicella at 20 weeks gestation	37	M	2300
39	506/76	a	2.70	17.40	—	41	F	2540
40	638/76	a	1.00	15.10	—	40	M	2920
41	644/76	a	1.40	10.90	—	38	F	3100
42	901/76	a	0.61	7.89	—	38	M	3000
43	1316/76	T.C.	1.62	13.18	Disproportion	39	F	3230
44	1438/76	T.C.	1.33	12.56	Left ovarian cyst	40	M	2620
45	1456/76	a	1.53	15.02	—	40	M	3500
46	47/77	T.C.	0.50	5.20	Previous neonatal death (birth trauma)	37	M	2260
47	140/77	a	0.94	11.90	—	40	F	3810
48	221/77	a	1.70	13.54	—	40	F	3180
49	289/77	a	1.31	10.40	—	42	F	2670
50	284/77	T.C.	1.88	14.81	Previous C. Section	40	F	3220
51	305/77	T.C.	0.47	5.07	Breech	38	F	2440
52	293/77	a	2.70	17.48	—	39	F	3320
53	329/77	T.C.	0.89	9.72	Breech	40	F	2590
54	315/77	T.C.	0.87	8.78	Fetal Distress	40	M	3750

a=High puncture of the membranes (Drew-Smythe catheter)
T.C.=Uterine puncture at caesarean section
Ext.=cases from hospitals outside the department of obstetrics and gynaecology of the University of Genova. All other cases are from the department of obstetrics and gynaecology of the University of Genova.

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Table 2. *Details of 30 pathological cases.*

Case N.	chart number/ year	amniotic fluid obtained by	miristic acid/ stearic acid ratio	palmitic acid/ stearic acid ratio	clinical notes	newborn		
						gestational age	sex	birth weight
1	998/74	a	1.40	13.82	Potential diabetes	40	F	3650
2	1159/74	a	3.10	25.07	Potential diabetes	39	M	3430
3	210/75	T.C.	0.30	3.20	Potential diabetes and epilepsy	39	F	2325
4	1213/75	T.C.	1.38	5.60	Potential diabetes	36	F	2100
5	1154/75	A	0.23	4.40	Diabetes Class B	33		
	same	T.C.	1.10	7.10		36	F	2200
6	Ext. 6	T.C.	0.32	3.23	Diabetes Class B	36	F	3450
7	Ext. 15	T.C.	0.50	4.25	Diabetes Class A	38	F	2250
8	126/76	T.C.	1.25	8.25	Diabetes Class A and breech	37	M	3150
9	324/76	T.C.	2.00	20.00	Potential diabetes and breech	40	F	2800
10	325/76	T.C.	1.00	10.60	Potential diabetes, disproportion	39	M	3040
11	303/76	T.C.	1.95	7.94	Diabetes Class C	35	M	2020
12	327/76	T.C.	0.89	7.83	Diabetes Class A	38	F	3700
13	330/76	a	2.40	19.50	Potential diabetes	39	F	3730
14	321/76	a	1.80	16.80	Potential diabetes	38	F	3140
15	437/76	a	1.04	6.75	Potential diabetes	40	F	3350
16	414/76	a	0.85	6.55	Latent diabetes and siphylis	39	M	4500
17	436/76	a	0.40	9.76	Potential diabetes	40	M	3980
18	750/76	a	0.83	10.30	Gestational diabetes	39	F	3160
19	1228/76	T.C.	0.64	5.10	Diabetes Class A	38	F	2810
20	1385/76	T.C.	0.92	7.52	Potential diabetes	40	F	3950
21	96/77	A	0.21	2.70	Gestational diabetes	34		
	same	T.C.	0.48	4.76		37	F	2650
22	183/77	T.C.	0.39	4.20	Diabetes Class B and PBSP	33	M	2650
23	318/77	T.C.	0.86	7.78	Potential diabetes	37	F	3380
24	476/75	A	0.26	2.40	Potential diabetes	33		
	same	a	0.38	5.10	Rh isoimmunisation	37	F	3250
25	Ext. 5	a	0.45	3.97	E.P.H. gestosis Anencephalus	37	F	2300
26	69/76	T.C.	0.21	2.94	Systemic lupus erythematosus E.P.H. gestosis Early neonatal death	33	M	770
27	1286/76	T.C.	0.74	6.81	E.P.H. gestosis	37	M	2660
28	277/77	T.C.	0.27	2.22	Essential hypertension Kidney stones (right)	30	M	1080
29	Ext. 9	T.C.	0.37	2.90	Placental separation, early neonatal death	34	F	1740
30	1273/76	T.C.	0.44	3.45	Fetal death at 37 weeks	36	M	2690

a = High puncture of the membrane (Drew-Smythe catheter) — T.C. = Uterine puncture at caesarean section — A = Amniocentesis — Ext. = cases from hospitalis outside the department of obstetrics and gynaecology of the University of Genova. All other cases are from the department of obstetrics and gynaecology of the University of Genova. — Classification of Diabetes: According to P. White as reported by Pescetto et al., 1977. — PBSP = Prognostic bad signs in pregnancy (Pedersen, 1971).

The method of preparation of the samples is the same as described by Castello et al. (1976), with the following two exceptions:

a) *centrifugation*: samples were centrifuged at room temperature at 3500 x g for 60 minutes.
b) *final concentration of the sample*: it was continued until the end volume was 0,2 ml; satisfactory chromatograms could be obtained by means of injections of 0,5 microliter of this fluid.

The whole procedure can be done on multiple samples at the same time.

The details of the method are the following:

The samples of amniotic fluid are immediately centrifuged at 3500 x g at room temperature for one hour and are then stored at −20° C.

The extraction procedure is as follows:

1. 10 ml of amniotic fluid are poured into a separatory funnel with 200 ml of chloroform/methanol mixture (130/70 v/v) and 40-50 ml of 0,9% aqueous NaCl.
2. The upper layer is discarded and the lower (organic) layer, containing the substances to be determined, is recovered.
3. The organic solvent is removed by heating at 140-150° C.
4. The residue is dissolved in 5 ml of 0,5N NaOH in methanol.
5. 4 ml of BF_3 (boron trifluoride) 20% in methanol are added and the mixture is boiled for two minutes.
6. The solution is poured into a separatory funnel along with 6-7 ml of saturated NaCl solution and 10 ml of petroleum ether.
7. The upper (organic) layer is recovered and concentrated to a volume of 0,2 ml.
8. The sample is ready for the gas-chromatographic analysis.

Details of gas-chromatographic analysis are summarized in table 3. A « Fractovap 2200 flame ionization detector C.E. » (C.E. = Carlo Erba) was employed, instead of the Varian aerograph 1520 used in the previous investigation.

By means of a suitable number of controls with the original procedure it could be shown that the modifications described above did not alter the end results nor the quality of the chromatograms, the outputs beeing perfectly comparable. Therefore it was decided to adopt this simplified procedure for all the present work.

Tab. 3. *Gas-chromatographic analysis of fatty acids in amniotic fluid; details of the procedure.*

Fractovap 2200 flame ionization detector (F.I.D.) « C.E. »					
Carrier gas	Nitrogen	1,6 Kg/cm$_2$		25 ml/min"	
F.I.D. Gases	air	1,5 Kg/cm$_2$			
	H_2	0,70 Kg/cm$_2$			
Column temperature	180° C	Injector temperature		300° C	
Glass column	Length	Outside diameter	Inside diameter	Liquid phase	Support
	m 2	mm 6	mm 2	Ethylen glycol succinate	Silanized on W. Chromosorb
Attenuation	Input			Output	
	x 10			x 32	
Chart speed	12"/hour				

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RESULTS AND DISCUSSION

As shown in table 1, in all 54 samples of amiotic fluid from normal pregnancies at term the value of palmitic acid/stearic acid ratio was constantly above 5 and in most cases it was much higher. Among the cases of table 1 there is a certain number of patients with a small-for-dates fetus, who were considered « normal » because there was no detectable indication of disease during pregnancy. As expected, none of the 54 babies listed in table 1 showed any respiratory disturbance and the neonatal course was uneventful, even when the birtweight was below 2.500 g.

Among the pathological cases listed in table 2, only 10 had a palmitic acid/stearic acid ratio of less than 5; in all the remaining cases the P/S ratio was 5 or above and none of these babies had respiratory difficulties in the neonatal period.

The details of the babies from the group with a P/S ratio of less than 5 are the following:

Case Ext. 5 was anencephalic and died within a few hours from birth.

Case 69/76 died of cardiac arrest one hour after delivery.

Case Ext. 9 was delivered by emergency caesarean section performed because of severe premature separation of the placenta (grade 2 according to Benson, 1971); the baby was severely asphyxiated and resuscitation efforts were not successful; death intervened a few minutes after delivery.

Case 1273/76 was the only baby who developed a moderate respiratory distress syndrome; however, progression of the disease could be avoided with appropriate management and the neonate was eventually discharged in good conditions.

The remaining six babies with a P/S ratio of less than 5 had no respiratory problems and were all discharged in good conditions.

Among the amniotic fluid samples with a P/S ratio of less than 5, the highest miristic acid/stearic acid ratio observed was 0,5 in only one case (the baby did not develop a RDS). There were a few cases with a miristic acid/stearic acid ratio inferior to 0,5 also in the group with a P/S ratio between 5 and 6, but there were none in the group with a P/S ratio of more than 6.

Therefore, we suggest that a M/S ratio of 0,5 may be used as subsidiary indicator of attained fetal lung maturation.

The low incidence of respiratory complications among the babies with a P/S ratio inferior to 5 may be explained by the prompt resuscitation measures and the subsequent expert neonatal intensive therapy. As a matter of facts, beside the obstetrician and the midwife, at the moment of all deliveries a physician-neonatologist was present in the delivery room and almost always there was also a physician-anaesthesiologist.

Consequently, on the basis of the present observations, the validity of a P/S ratio in amniotic fluid of 5 or more can be confirmed without exceptions as indicator of a satisfactory fetal lung maturity. Furthermore, when expert and efficient neonatal care is available, in the presence of a P/S ratio in amniotic fluid between 5 and 4 the risk of neonatal death following severe RDS does not seem to be of such magnitude to exclude planned pre-term delivery in selected cases with urgent indications.

It must be pointed out, however, that in order to obtain reliable diagnostic indications painstaking attention to technical details is necessary. As already pointed out ([5,6]) proper centrifugation of the amniotic fluid samples is essential in order to remove all particulate contaminants (especially fragments of vernix

caseosa) which could change completely the end results of fatty acids analysis. The recognition that centrifugation at 3.500 x g at room temperature can be substitued for centrifugation at 20.000 x g for 40 minutes at low temperature ($<5°C$) as originally suggested ([3]) further increases the availability of the present technique.

SUMMARY

A simplified method for gas-chromatographic determination of fatty acids in amniotic fluid is described. The present results on a series of normal and pathological cases further support the conclusions of other investigations that in amniotic fluid a palmitic acid/stearic acid ratio equal or greater than 5 is reliable indicator of attained fetal lung maturity.

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Circadian rhythm of plasma oestriol and urinary oestriol in pregnancy at term

by

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The use of plasma hormonal analyses as a means for establishing the functioning and condition of the foeto-placental unit is now widespread.

Among the various hormones of foeto-placental origin whose use has been suggested for the monitoring of pregnancy, the most reliable nowadays seems to be oestriol. Its levels in the plasma and urine behave in such a way as to provide a sufficiently safe and effective index within the scope of a surveillance programme, as complete as possible, in the last weeks of pregnancy ([1]), also because

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